Supplementary materials and methods

Plasmids

MSCV-MPLW515L-IRES-GFP retroviral construct was kindly provided by Dr. Ross Levine (MSKCC). Glycerol stocks of pLKO.1 Human CDK6 shRNA TRCN0000000486 (sh486) and pLKO.1 mouse CDK6 shRNAs TRCN0000023150 (sh50) and TRCN0000023153 (sh53) were purchased from Dharmacon.

In vitro knockdown, cell proliferation and apoptosis assays

For knockdown of CDK6, cells were transduced with the lentiviruses expressing CDK6 shRNA or scramble (control) shRNA and infected cells were selected using puromycin (2μg/mL). Cell proliferation was assessed every day over 4 to 5 days by viable cell counts using trypan blue exclusion. Experiments were replicated at least three times. To determine apoptosis, cells were cultured in the presence of DMSO, Palbociclib, Ruxolitinib or Palbociclib plus Ruxolitinib. Apoptosis assay was performed by Annexin V staining and evaluated by flow cytometry.

Blood and tissue analysis

Peripheral blood counts were determined using Hemavet 950FS (Drew Scientific). For histopathologic analysis, mouse tissue specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections (4 μm) were stained with reticulin stain.

Colony-forming assays

BM (2 X10^4) cells were plated in duplicates in cytokine-supplemented complete methylcellulose medium (MethoCult M3434; StemCell Technologies). Granulocyte-macrophage colony-forming units (CFU-GM) colonies were scored on day 7. CD34+ cells (2 X10^3) from MF patient or healthy control were plated in duplicates in cytokine-supplemented complete methylcellulose

medium (MethoCult H4034; StemCell Technologies) in the presence of DMSO, Palbociclib, Ruxolitinib or Palbociclib/Ruxolitinib combination. Colonies were scored on day 14.

Flow cytometry

Single-cell suspensions were prepared from BM and spleens, and red cells were lysed with red cell lysis solution. Cells were washed and resuspended in PBS plus 2% FBS and stained for 20 minutes on ice with directly conjugated (either PE or APC) monoclonal antibodies specific for CD41, CD61, Mac-1or Gr-1. For LSK (Lin-Sca1+c-kit+) cells analysis, cells were stained for 30 to 60 minutes on ice with antibodies against c-Kit, Sca-1, Flk2 (CD135), CD34, CD16/32 (FcγR II/III), and antibodies against lineage (Lin) markers including CD3, CD4, CD8, CD19, B220, Gr-1, Ter119, and IL-7R (CD127). All antibodies were purchased from eBioscience, or BioLegend. Flow cytometry was performed with an LSRII (BD Biosciences) and analyzed using FlowJo software.

Enzyme-linked immunosorbent assay (ELISA)

TGF-β1, IL-6 and IL-1β levels in the serum of mice were determined using ELISA kits (R&D Systems) according to the manufacturer's protocols.

Supplementary Figure legends

Supplementary Figure 1. Ruxolitinib treatment does not affect CDK6 expression. A and **B,** Immunoblots showing reduced phosphorylation of STAT5 but no change in CDK6 protein levels in JAK2V617F positive HEL cells (**A**) and MF PBMC (**B**) upon Ruxolitinib treatment compared with vehicle (DMSO) treatment. **C,** In vivo treatment of Ruxolitinib resulted in marked inhibition of STAT5 phosphorylation but little or no change in CDK6 protein expression in $Jak2^{VF/VF}$ mice BM. β-actin served as a loading control.

Supplementary Figure 2. Knockdown of CDK6 significantly reduces proliferation in hematopoietic cells expressing JAK2V617F. A-D, BA/F3-EpoR-JAK2V617F (**A**), BA/F3-EpoR (**B**), SET-2 (**C**) and HEL (**D**) cells were transduced with lentiviral scramble shRNA (control) or CDK6 shRNA (KD_1 and KD_2) and selected using puromycin. Cell proliferation was determined by viable cell counts every 24 hours for 5 days by trypan blue exclusion method. Note that knockdown of CDK6 significantly inhibited proliferation of JAK2V617F-positive BA/F3-EpoR-JAK2V617F, SET-2 and HEL cells but did not affect the proliferation of wild-type JAK2-expressing BA/F3-EpoR cells (*p<0.05; **p<0.005, ***p<0.0005; Student's t-test). Data from three independent experiments are presented. Immunoblot showing effective knockdown of CDK6 in respective cell lines. β-actin served as a loading control.

Supplementary Figure 3. Palbociclib treatment induces apoptosis in cells expressing JAK2V617F. JAK2V617F-positive BA/F3-EpoR-JAK2V617F cells were treated with vehicle (DMSO) or Palbociclib (0.125-0.5μM) for 3 days. Annexin V/propidium iodide (PI) staining followed by flow cytometric analysis was performed to measure apoptosis. Representative dot plots and bar graphs shows Palbociclib treatment (0.5μM) significantly increased apoptosis in BA/F3-EpoR-JAK2V617F cells. (*p<0.05).

Supplementary Figure 4. Effects of Palbociclib/Ruxolitinib treatment on healthy control hematopoietic progenitor colony formation. A and **B,** Healthy control CD34+ cells were plated in complete methylcellulose medium supplemented with cytokines in the presence of DMSO, Palbociclib alone or in combination with Ruxolitinib. Data are represented in bar graphs as mean ± SEM (n= 4; *p<0.05; ***p<0.0005; **** p<0.00005; Student's t-test).

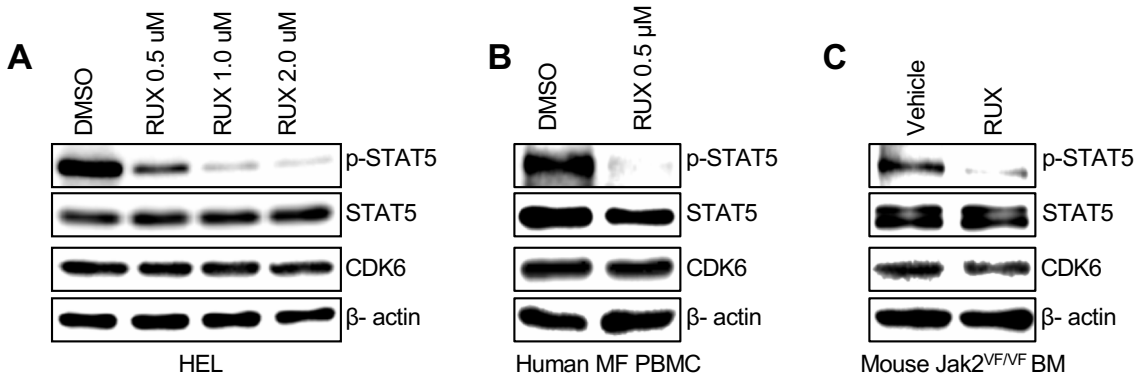
Supplementary Figure 5. Effects of Palbociclib/Ruxolitinib treatment on gene expression in Jak2$^{VF/VF}$ mice LSK and SET-2 cells. A, GSEA analysis of the RNA-sequencing data from Palbociclib/Ruxolitinib treated Jak2$^{VF/VF}$ mice LSK cells compared with Ruxolitinib treated Jak2$^{VF/VF}$ mice LSK. Enrichment plots of selected gene sets with normalized enrichment score (NES) and false discovery rate (FDR) are shown. **B,** Microarray gene expression data analysis of MPN patient dataset (GSE54644) show significantly elevated expression of AURKA, AURKB and HMGA2 in granulocytes of MF patients compared with healthy controls. (Healthy controls = 11, MF=18). (**p<0.005, ***p<0.0005). **C,** Relative expression of AURKA, AURKB, and HMGA2 mRNA was determined in SET-2 cells treated with vehicle, Palbociclib, Ruxolitinib or Palbociclib plus Ruxolitinib by RT-qPCR and normalized with HPRT expression. Data from three independent experiments are shown in bar graphs as mean ± SEM (*p<0.05, **p<0.005).

Supplementary Figure 6. Palbociclib treatment alters cell signaling in SET-2 cells. Immunoblot analysis shows decreased phosphorylation of Rb and p65 subunit of NF-κB and reduced expression of HMGA2, AURKA and AURKB in SET-2 cells by treatment of Palbociclib or Palbociclib/Ruxolitinib combination. β-actin was used as a loading control.

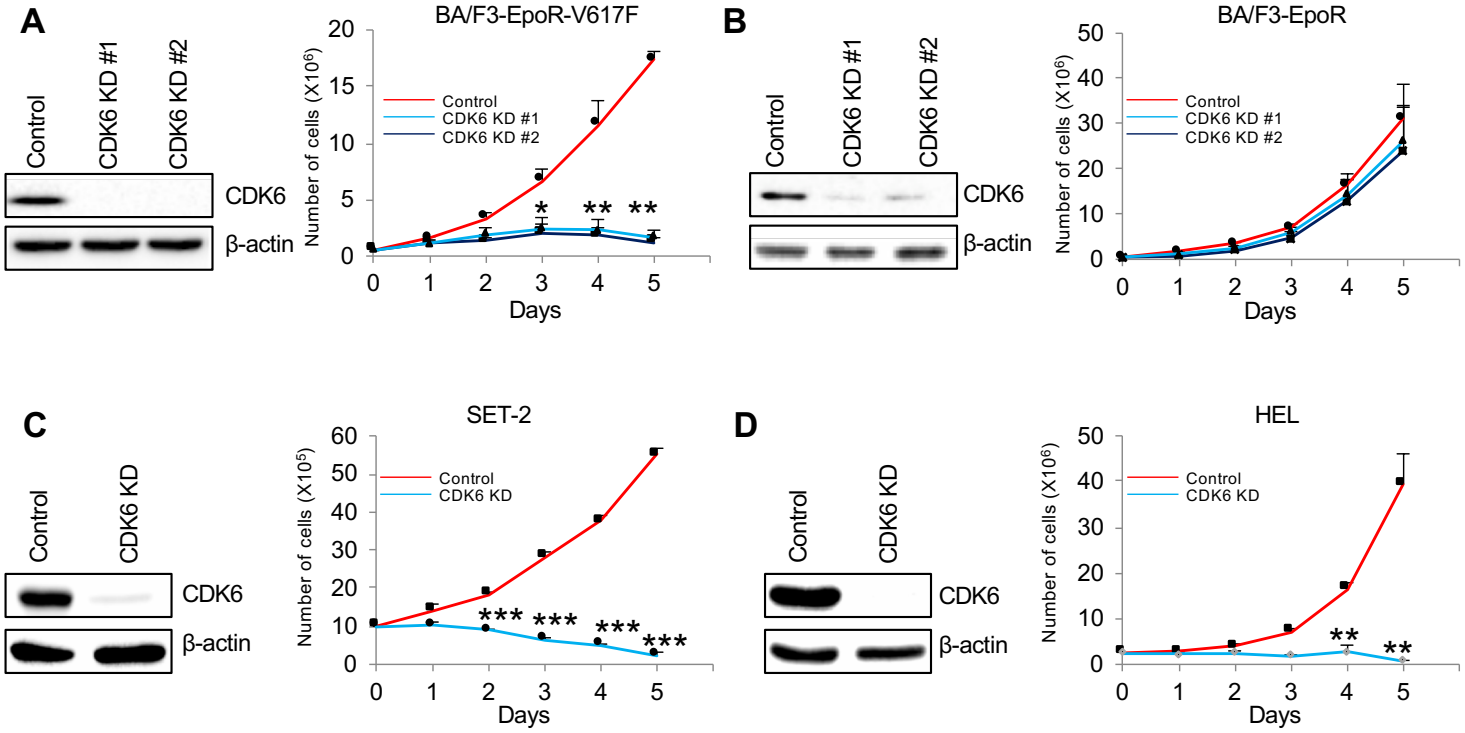
Supplementary Figure 7. Effects of Palbociclib/Ruxolitinib treatment on IL-6 and IL-1β levels in Jak2$^{VF/VF}$ mice. A and **B,** Serum IL-6 (**A**) and IL-1β (**B**) levels in Jak2$^{VF/VF}$ mice

treated with vehicle, Palbociclib, Ruxolitinib and combination were assessed by ELISA (n = 4-6) (*p<0.05; **p<0.005; Student's t-test).

Supplementary Figure 8. Effect of Ruxolitinib treatment on TGF-β1 induced Collagen expression in BM MSC. Stimulation with TGF-β1 (50ng/ml) significantly increased Collagen I and Collagen III expression in BM MSC. Ruxolitinib (0.125μM) treatment in presence of TGF-β1 (50ng/ml) did not alter Collagen (I and III) expression. The mRNA expression was assessed by RT-qPCR and normalized by Hprt. Data are shown in bar graphs as mean ± SEM (n=3; ***p<0.0005; Student's t-test).



Supplementary Figure 1



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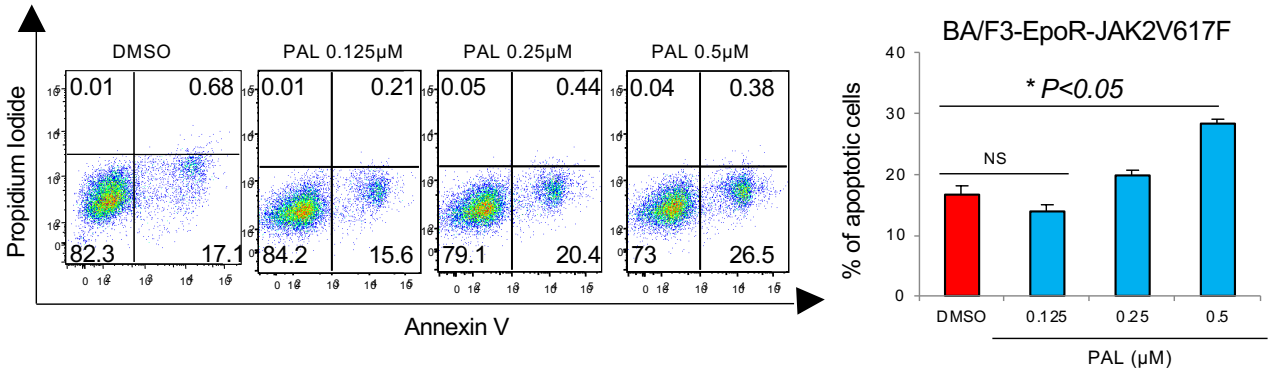
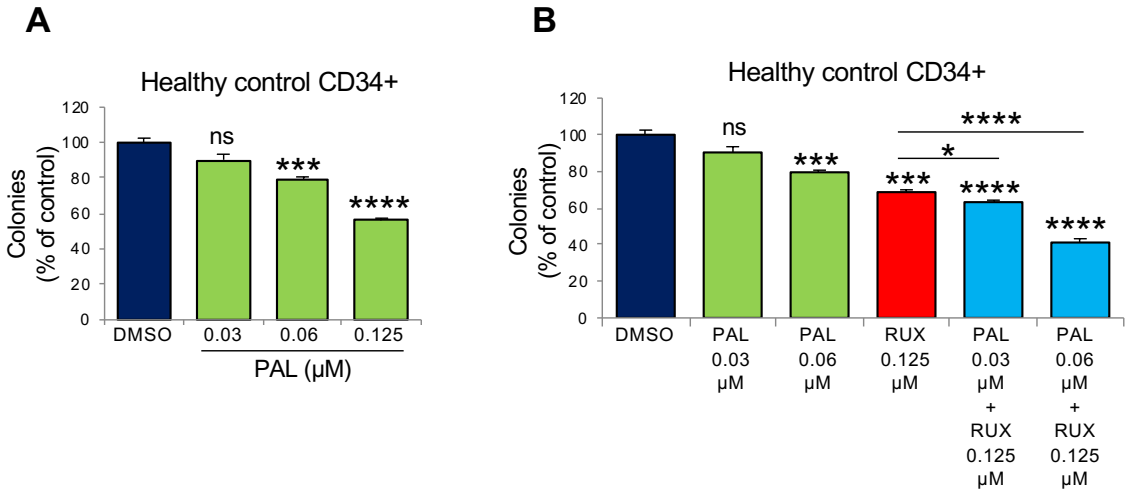
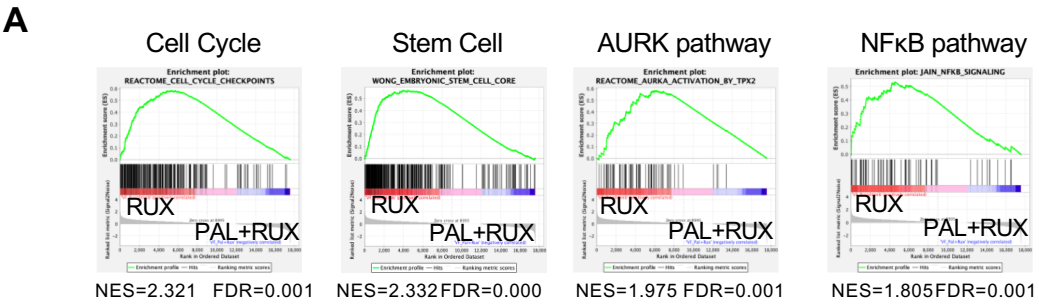
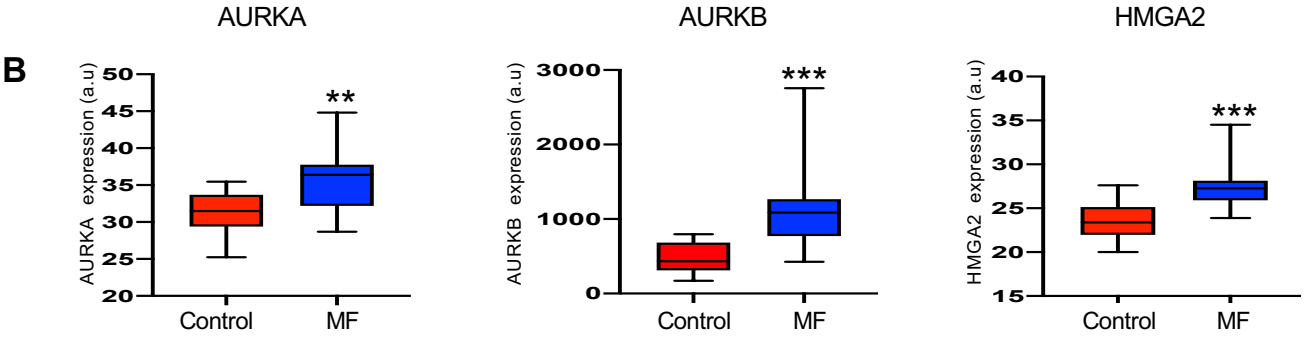


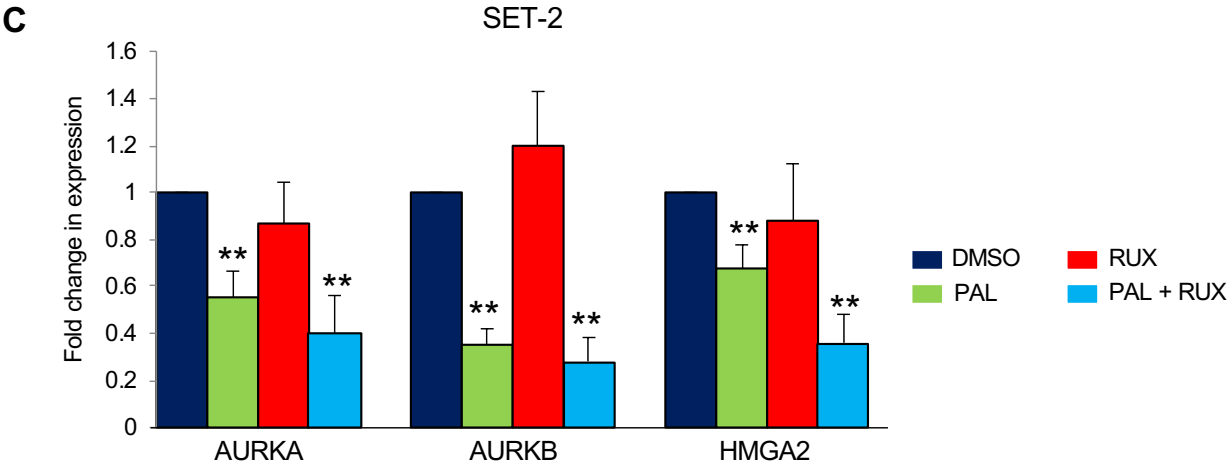
Supplementary Figure 3



Supplementary Figure 4







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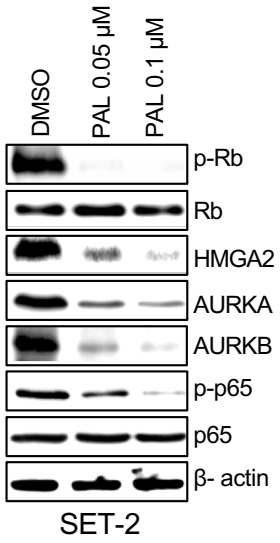

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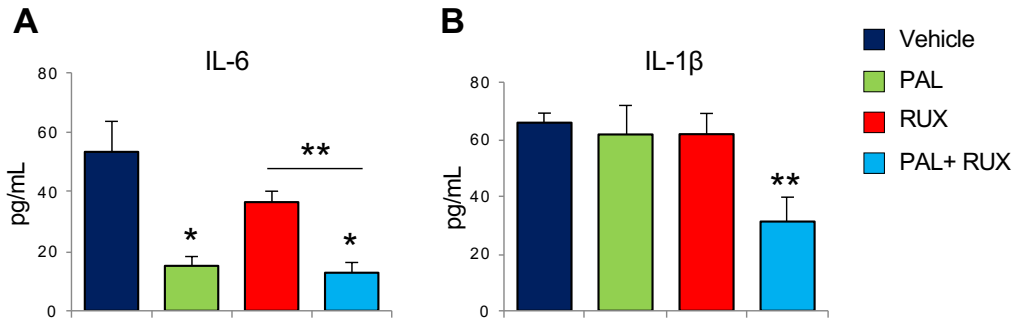
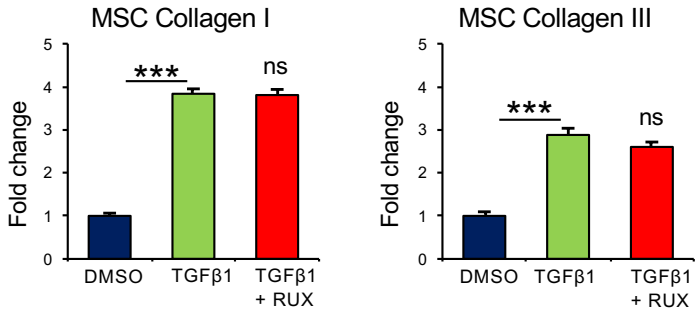


Supplementary Figure 7



Supplementary Figure 8

Supplementary Table 1. Primers used for mice real-time quantitative PCR

mouse *Col1a1* Forward	TGGTCCCTCTGGAAATGCTG
mouse *Col1a1* Reverse	AACTTCACCAGGACGTCCAG
mouse *Col3a1* Forward	TGACAGAGGAGAAACTGGCC
mouse *Col3a1* Reverse	GCCATTAGAGCCACGTTCAC
mouse *Aurka* Forward	TGAGTTCCTAGTGGGGATGC
mouse *Aurka* Reverse	CCGCTAGTGTTAGCCTTTGG
mouse *Snail1* Forward	CCACTGCAACCGTGCTTTT
mouse *Snail1* Reverse	CACATCCGAGTGGGTTTGG
mouse *Hmga2* Forward	ACCCAAAGGCAGCAAAAAC
mouse *Hmga2* Reverse	GCAGGCTTCTTCTGAACGAC
mouse *Aurkb* Forward	GAAGAAGAGCCGTTTCATCG
mouse *Aurkb* Reverse	GGATGTTGGGATGTTTCAGG
mouse *Cdk6* Forward	CAACGTGGTCAGGTTGTTTG
mouse *Cdk6* Reverse	AGACCTCGGAGAAGCTGAAAC
mouse *αSMA* Forward	CTGACAGAGGCACCACTGAA
mouse *αSMA* Reverse	CATCTCCAGAGTCCAGCACA
mouse *18S* Forward	CGCCGCTAGAGGTGAAATTC
mouse *18S* Reverse	TTGGCAAATGCTTTCGCTC
mouse *Hprt1* Forward	CAACGGGGGACATAAAAGTTATTGGTGGA
mouse *Hprt1* Reverse	TGCAACCTTAACCATTTTGGGGCTGT
mouse *Gapdh* Forward	ACTCCACTCACGGCAAATTC
mouse *Gapdh* Reverse	TCTCCATGGTGGTGAAGACA

Supplementary Table 2. Primers used for human real-time quantitative PCR

human *CDK6* Forward	TGTTTCAGCTTCTCCGAGGTC
human *CDK6* Reverse	ACTATAGATGCGGGCAAGGC
human *HMGA2* Forward	GAAGCAGCAGCAAGAACCA
human *HMGA2* Reverse	TTGTGGCCATTTCCTAGGTC
human *AURKA* Forward	GCAGATTTTGGGTGGTCAGT
human *AURKA* Reverse	AAGGCTCCAGAGATCCACCT
human *AURKB* Forward	GGGAGAGCTGAAGATTGCTG
human *AURKB* Reverse	GCACCACAGATCCACCTTCT
human *HPRT1* Forward	TGCAGACTTTGCTTTCCTTGGTCAGG
human *HPRT1* Reverse	CCAACACTTCGTGGGGTCCTTTTCA
human *GAPDH* Forward	GAGTCAACGGATTTGGTCGT
human *GAPDH* Reverse	GACAAGCTTCCCGTTCTCAG
human *18S* Forward	CGCCGCTAGAGGTGAAATTC
human *18S* Reverse	TTGGCAAATGCTTTCGCTC